Photochemical Lesions in the Primate Retina under Conditions of Elevated Blood Oxygen

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Under conditions of nonthermal radiant exposure to blue light (440 nm) the primate retina can suffer photic injury by a mechanism that must be photochemical in nature. We have examined the effects of elevated blood oxygen (pO_2 of 270 mmHg) on the retinal photosensitivity to blue light in two macaque monkeys by histologic analysis of 12 lesions at 1 to 57 days after irradiation. The retinal image diameter from a xenon arc lamp source was 1 mm, the duration of exposure was 100 sec, and the radiant exposures ranged from 11 to 36 J/cm^2. When blood oxygenation is not elevated experimentally, the threshold radiant exposure for a blue light lesion to be visible funduscopically at 2 days postexposure is about 30 J/cm^2. At a high blood pO_2 level, a radiant exposure of only 11 J/cm^2 gave a funduscopically visible lesion at 1-day postexposure. This large increase in retinal sensitivity to blue light damage appears to be due to photodynamic action. The only direct effect of elevated blood pO_2 on the retina observed histologically was the presence of numerous granules in the cells of the retinal pigment epithelium (RPE). However, there was no apparent histopathology associated with the elevation of blood pO_2 alone. Analysis of the various photic lesions showed only moderate damage to the neural retina, but a strong response was seen in the RPE. This is the histopathologic pattern of a typical blue light lesion shown in previous studies but more severe. So the effect of elevated blood O_2 is to increase retinal sensitivity to photic damage, to lower the damage threshold, and to increase the severity of damage at a given radiant exposure. The status of lesions at 23 and 57 days postexposure suggests that such injuries are repairable. Invest Ophthalmol Vis Sci 25:893–898, 1984

Nonthermal focal radiant exposure of the macaque retina to blue light (440 nm) can produce localized photic injury by a mechanism that is photochemical in nature. Previous studies employing serial fundus photography,[1,2] histologic analysis,[3,4] and visual acuity testing[5] have served to characterize the behavior of such focal blue light lesions. However, the basic mechanisms of the photochemical damage are unknown. A recent study of the relationship between the exposure threshold for blue light lesions and the level of blood oxygenation[6] has indicated that retinal photosensitivity is enhanced by oxygen. The present study characterizes, by histologic analysis, photochemical lesions in the macaque retina under conditions of elevated blood oxygen.

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Materials and Methods

This study was carried out using two macaque monkeys. Our damage thresholds are essentially all-or-no responses (ED_{100} rather than ED_{50}), obviating the need for statistical designs using several animals. The radiation source was a 2500 W xenon lamp optical system using a narrow waveband of 440 ± 5 nm. The retinal image diameter was 1 mm, and the retinal radiant exposures ranged from 11 to 36 J/cm^2. All the experimental procedures for irradiating the monkey retina have been described in detail elsewhere.[2,3,6] Oxygenation of the animals was accomplished under anesthesia by means of an endotracheal tube attached to a nonrebreathing apparatus equipped with separate inhalation and exhalation valves with attached gas bag, which was kept slightly above atmospheric pressure using a needle valve regulator. A previously prepared tank mixture with an 80/20 ratio of O_2/N_2 was used. Arterial (femoral) blood samples were taken before oxygenation and at 30 min after oxygenation. These samples were analyzed immediately for pO_2 levels. The blue light exposures were performed immediately after 30 min of oxygenation.

The histologic data are qualitative by design. Having focal lesions surrounded by normal tissue, we compare the damaged tissue within the exposed area with that

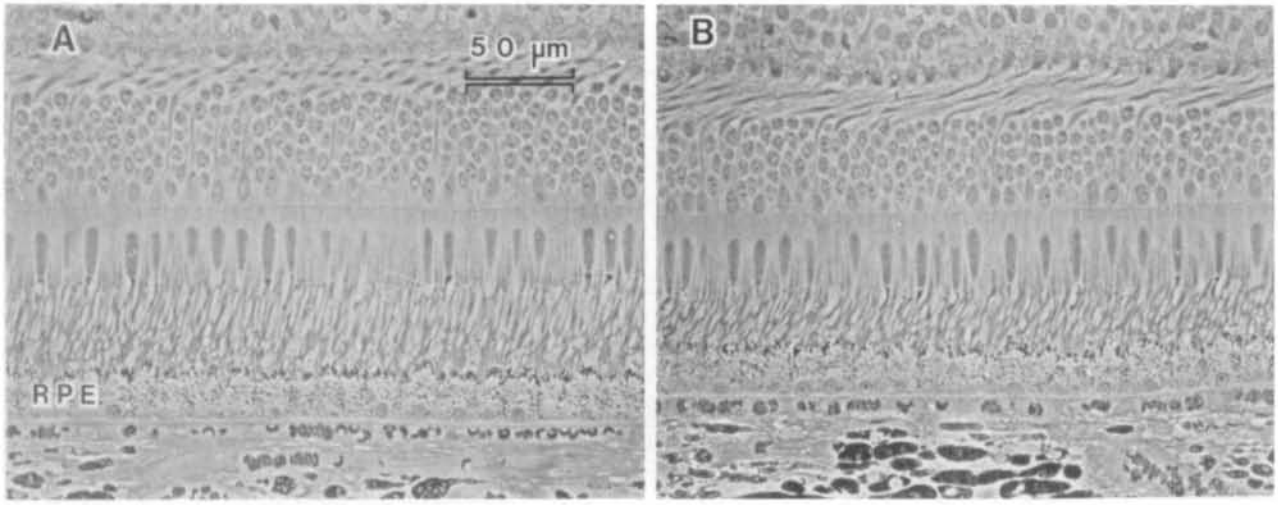

Fig. 1. Effects of elevated blood pO_2 level alone. **A**, An example of unirradiated tissue from the first monkey. Numerous granules are present in retinal pigment epithelial cells (RPE). **B**, An example of unirradiated tissue from the second monkey. The appearance of the neural retina and RPE is essentially identical to **A**.

of the unexposed control tissue. The material for histologic analysis consisted of three tissue samples containing 12 photic lesions from two eyes (the right eye of each monkey). From the first monkey a 4 × 8 mm sample of the fundus containing unirradiated tissue and four photic lesions fixed at 1 day postexposure was taken. The range of radiant exposures was 11–36 J/cm^2. In addition, a 2-mm trephine sample contained two lesions fixed at 57 days postexposure. For both of these lesions the radiant exposure was 20 J/cm^2. From the second monkey a 5 × 8 mm sample of the fundus contained unirradiated tissue and six photic lesions. At the time of preparation, four of the lesions were fixed at 2 days postexposure and two at 23 days postexposure. The range of radiant exposures was 15–33 J/cm^2.

After enucleation, the eye was slit at the base of the cornea and immersed in 10% acrolein in 0.1 M cacodylate buffer (pH 7.3) for 30 min at room temperature. Then the eye was transected near the equator and the appropriate tissue samples were cut from the fundus. The samples then were immersed in the acrolein fixative for an additional 30 min. The samples were rinsed in cacodylate buffer, postfixed in 2% OsO_4 in 0.1 M cacodylate buffer for 1 hr at room temperature, rinsed again in buffer, and dehydrated in an acetone series (50, 75, 95, 100, 100%) for 10 min at each step. The samples were embedded in Ladd ultralow viscosity plastic in flat molds. The blocks were cut on an ultramicrotome to give 1.5–2.5 μm sections (unstained) for phase contrast light microscopy. More than 1600 sections were used for the histologic analysis.

In conducting these experiments, the investigators adhered to the *Guide for Laboratory Animal Facilities and Care* of the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

Results

The retinal tissue samples contained a large proportion of unirradiated tissue which served as an experimental control for the direct effects of elevated blood pO_2 alone. Examples of control retinal tissue from both monkeys (Fig. 1) were essentially identical in appearance. There was no apparent histopathology associated with increased oxygenation alone. The only direct effect of elevated blood pO_2 on the retina observed histologically was the presence of numerous granules in the cells of the retinal pigment epithelium (RPE). The neural retina showed no apparent abnormalities.

Normally, when blood pO_2 is not experimentally elevated, the threshold radiant exposure for a blue light lesion to be funduscopically visible at 2 days postexposure is about 30 J/cm^2. Generally blue light damage is not detectable histologically unless it is funduscopically visible. The right eye of the first monkey received a series of four exposures ranging from 11 to 36 J/cm^2 that were funduscopically visible at 1 day postexposure and were analyzed histologically (Fig. 2). Even at the lowest radiant exposure photic damage was clearly evident (Fig. 2A). In all four lesions, the damage to the neural retina was moderate. Damaged cone ellipsoids were prominent. Some pyknotic nuclei were observed in the outer nuclear layer (Figs. 2A, D). An

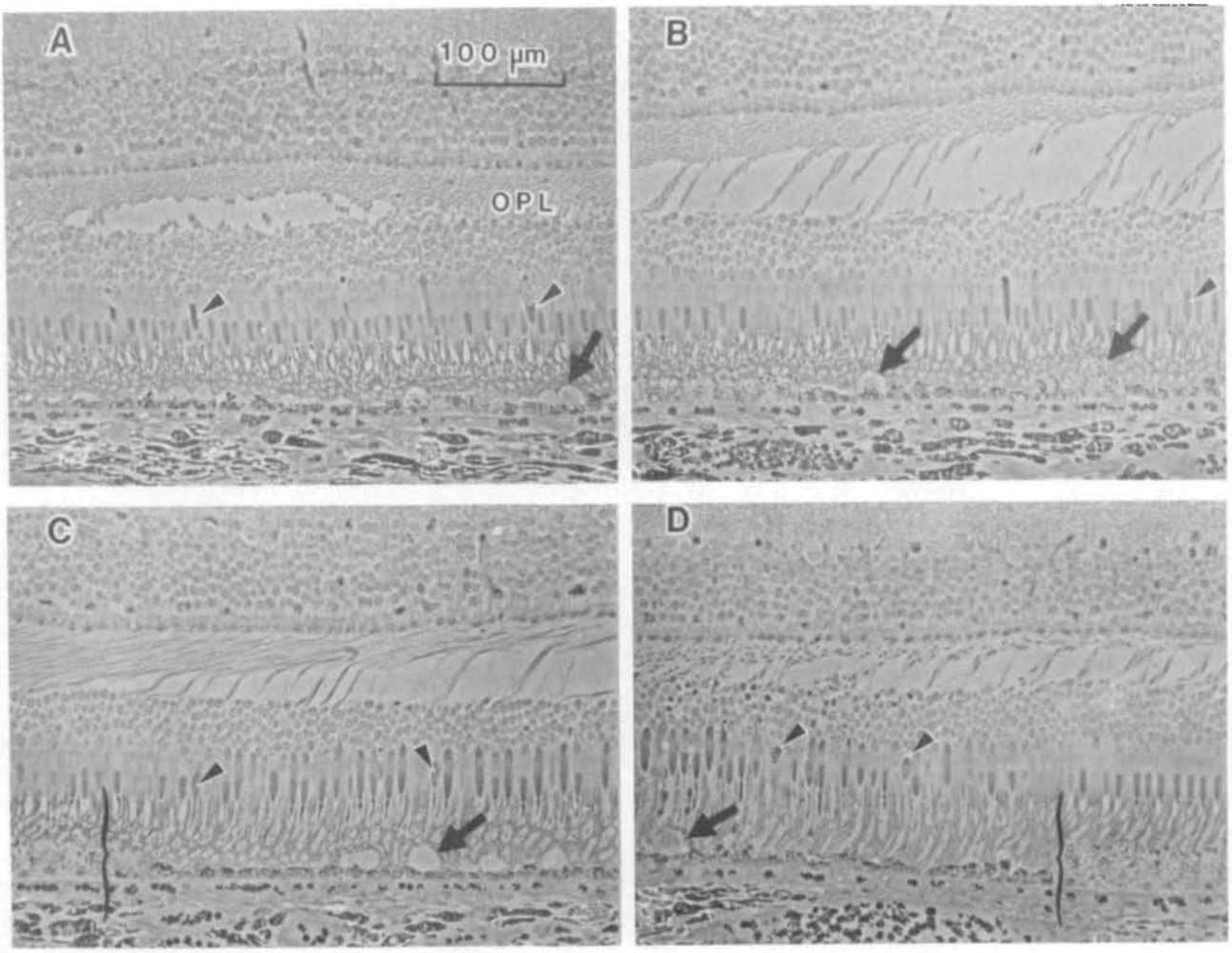

Fig. 2. Four photic lesions from the first monkey fixed at 1 day postexposure. **A**, Radiant exposure was 11 J/cm^2. RPE cells showed flattening, swelling (arrow), and clumping of melanin granules. Some damaged cone ellipsoids are apparent (arrowheads), and there is an abnormal space in the outer plexiform layer (OPL). **B**, Radiant exposure was 21 J/cm^2. Some RPE cells showed prominent swelling (arrows). A damaged cone ellipsoid is indicated by an arrowhead. There is a large space in the OPL. **C**, Radiant exposure was 30 J/cm^2. The bracket marks a border of the lesion. Flattening of the RPE cells is evident, and there are several cyst-like spaces (arrow). Some damaged cone ellipsoids are indicated by arrowheads, and there is a large space in the OPL. **D**, Radiant exposure was 36 J/cm^2. The bracket marks a border of the lesion. A swollen RPE cell (arrow) and damaged cone ellipsoids (arrowheads) are indicated. There is an abnormal space in the OPL.

abnormal space of variable extent was observed in the outer plexiform layer (OPL) of each lesion. Such spaces were not seen in unirradiated areas of the tissue samples. The RPE of each lesion showed the greatest injury. The cells tended to flatten against Bruch's membrane (Figs. 2C, D) and in some cases to swell (Figs. 2A, B); and melanin granules were clumped in the cells. Occasionally, cyst-like spaces were seen in the subretinal space (Fig. 2C), which may represent pockets of edema fluid or blisters from highly swollen cells.

The right eye of the second monkey received a series of four exposures ranging from 15 to 33 J/cm^2 that were analyzed at 2 days postexposure (Fig. 3). All the types of damage to the neural retina and RPE seen at 1 day were also evident at 2 days after exposure. In addition, there were numerous macrophages present in the subretinal space. Normally, if blood pO_2 were not elevated, the radiant exposures shown in Figures 3A, B would have shown no histologic damage.

The fundus sample from the second monkey contained two additional photic lesions fixed at 23 days postexposure. At 17 J/cm^2 (Fig. 4A) the damage appeared moderate, with damaged cone ellipsoids and RPE cells, and with macrophages in the subretinal space. It is likely that at 1 day and 2 days postexposure, this area would have resembled the lesions in Figs. 1B, 2A, B. At 30 J/cm^2 (Fig. 4B) the apparent damage was more extensive. The photoreceptor outer segments

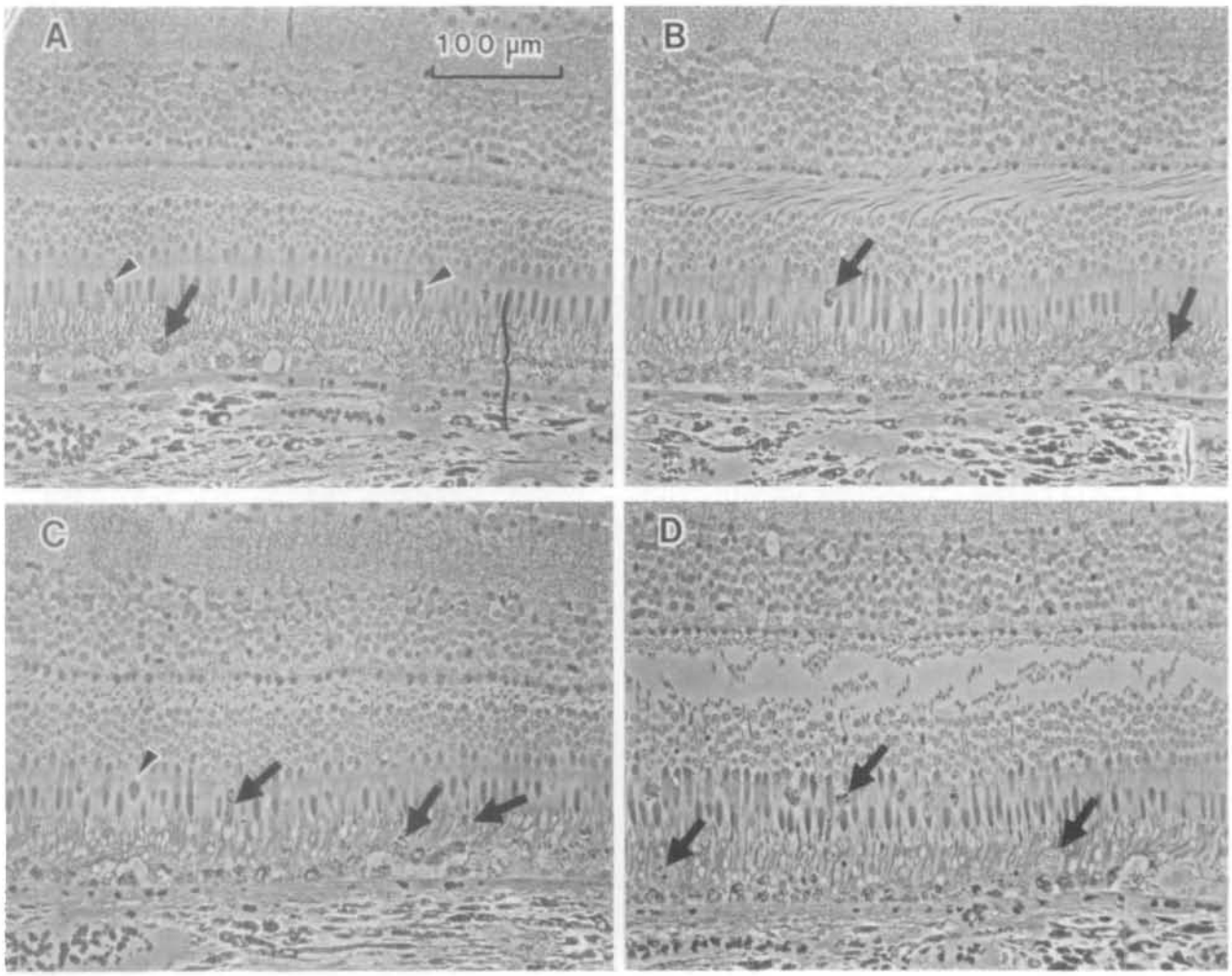

Fig. 3. Four photic lesions from the second monkey fixed at 2 days postexposure. **A**, Radiant exposure was 15 J/cm^2. The bracket marks a border of the lesion. A macrophage in the subretinal space (arrow) and some damaged cone ellipsoids (arrowheads) are indicated. **B**, Radiant exposure was 18 J/cm^2. Some macrophages in the subretinal space are shown at arrows. **C**, Radiant exposure was 26 J/cm^2. Some macrophages (arrows) and a damaged cone ellipsoid (arrowhead) are indicated. **D**, Radiant exposure was 33 J/cm^2. There are numerous macrophages in the subretinal space (arrows) and a large space in the OPL.

were short and disoriented, the RPE was very hypopigmented, and macrophages in the subretinal space were very numerous. This lesion represents the progression from an earlier state probably like the lesions in Figures 2C and 3D.

A trephine sample from the first monkey contained two lesions fixed at 57 days postexposure (Fig. 5). For both of them, the radiant exposure was 20 J/cm^2. At earlier stages, these areas of retina probably resembled the lesions shown in Figs. 2B, 3B, and 4A. At nearly 2 months after exposure, these lesions were still histologically evident (Fig. 5), but a process of repair seems to have occurred.

Discussion

In the unirradiated areas of tissue from both monkeys, the RPE cells contained numerous granules in the cytoplasm, but otherwise these cells looked normal. The neural retina and choroid showed no histologic changes due to elevated blood pO_2. The significance of the cytoplasmic granules in RPE cells is not clear. Ultrastructural analysis should provide the means to characterize the granules and, perhaps, to explain their relationship with oxygenation of the tissue. Nevertheless, in the context of these experiments, the elevation of blood pO_2 was not in itself toxic to the retina, at least by histopathologic criteria.

The histologic observations on the photic lesions at 1-day postexposure are particularly interesting. Normally, when blood oxygenation is not experimentally increased, the threshold radiant exposure for a funduscopically visible blue light lesion is about 30 J/cm^2.[2-4] In these experiments, when blood pO_2 was brought to 270 mmHg, a radiant exposure of only 11 J/cm^2 gave a funduscopically visible lesion at 1-day

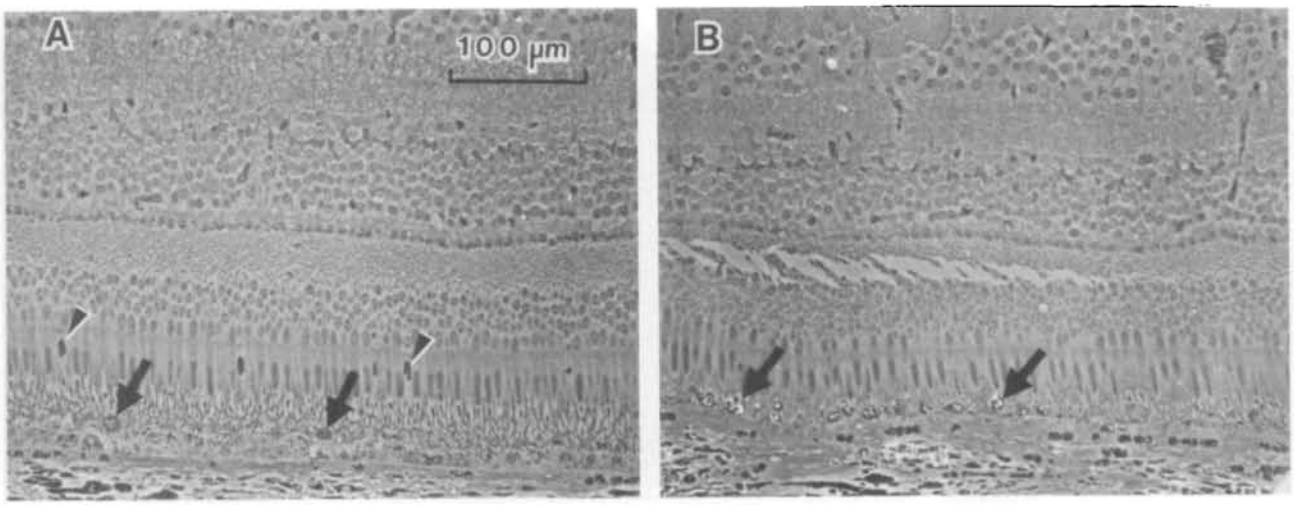

Fig. 4. Two photic lesions from the second monkey fixed at 23 days postexposure. **A**, Radiant exposure was 17 J/cm^2. Macrophages (arrows) and damaged cone ellipsoids (arrowheads) are indicated. **B**, Radiant exposure was 30 J/cm^2. RPE is very hypopigmented with numerous macrophages (arrows) in the subretinal space. Photoreceptor outer segments are short, and there is a space in the OPL.

postexposure. This large increase in retinal sensitivity to blue light damage is probably due to photodynamic action. The histology of the oxygen enhanced blue light lesions at 1 day and 2 days postexposure (Figs. 2, 3) shows features that are not observed in typical, near-threshold, blue light lesions.[3,4] The rounding up and flattening out of RPE cells, clumping of melanin granules, and tendency for cell swelling indicate more severe damage to the RPE. The damage to photoreceptor cells (pyknotic nuclei and cone ellipsoids) may be largely a consequence of the severe RPE damage, but it is difficult to distinguish primary and secondary photoreceptor cell damage. The large spaces distorting the outer plexiform layer (OPL) look like a separation (mechanical) artifact of specimen preparation. Because this OPL distortion occurs in several of the lesion sites of the tissue samples, but not in any of the regions of control tissue, it likely represents a feature of the lesions. Probably it indicates edematous swelling in the OPL. Such localized retinal swelling might explain the unusual funduscopic appearance of these lesions that has been described elsewhere.[6] Other remarkable features of the oxygen enhanced blue light lesions at 1 and 2 days postexposure are that the subretinal space did not show large distortions, that the photoreceptor outer segments did not show large-scale destruction, and that the inner layers of the neural retina appeared completely normal, including the blood vessels. The choriocapillaris and the choroid appeared unaffected by the treatment.

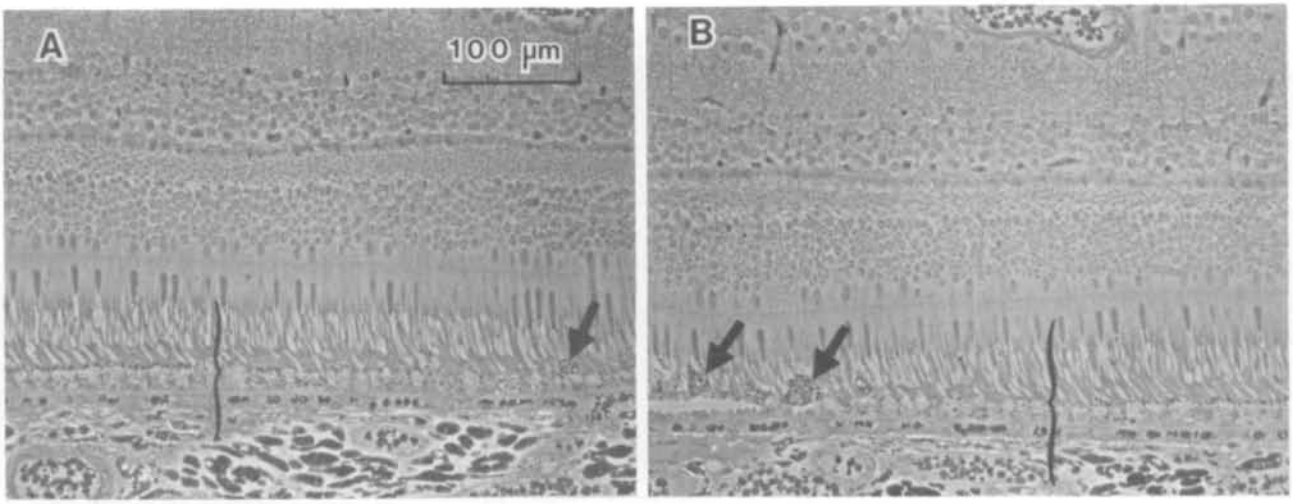

Fig. 5. Two photic lesions from the first monkey fixed at 57 days postexposure. The radiant exposure for both was 20 J/cm^2. **A**, The bracket marks a border of the lesion, and a macrophage in the subretinal space is indicated by an arrow. **B**, The bracket marks a border of the lesion, and macrophages (arrows) are present in the subretinal space. (The space in the RPE at the left is an artifact.)

At 23 days and 57 days postexposure, the histologic appearance of the lesions (Figs. 4, 5) indicate that the oxygen enhanced blue light lesions heal much as do typical blue light lesions,[3] but the recovery process may be somewhat prolonged. Perhaps this is a reflection of the increased severity of the RPE damage.

The work of Riva et al,[7] using laser Doppler shift measurements to show lowered retinal blood flow in human subjects when 100% O_2 is breathed, indicates the possibility that retinal blood flow might have been reduced in the monkey eyes during the exposures. Histologically there was no sign of damage to the inner layers of the retina in any of the lesions. The effects of oxygenation plus blue light were observed primarily in the RPE, adjacent to the choroidal blood supply. There is no evidence to indicate that choroidal blood flow is reduced by elevated pO_2, and it is likely that the RPE and outer retina were highly oxygenated via the choroidal circulation during the blue light exposures.

Based on this study and on previous reports,[3,4,6] we may draw some conclusions about retinal photochemical lesions. Under conditions of elevated blood oxygen the blue light damage shows the histopathologic pattern of a typical blue light lesion; but it is more severe, based on the rapid (1 day instead of 2 days) and striking morphologic changes (flattening out and swelling) in the RPE cells. The effect of elevated blood pO_2 is to increase retinal photosensitivity, to lower the damage threshold, and to increase the severity of damage at a given radiant exposure. The oxygen enhancement of blue light damage appears to be due to photodynamic action.

Key words: monkey, oxygen, photochemical lesion, pigment epithelium, retina

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